

1. (Amended) A method of treating disturbances in iron distribution in a patient suffering from heart disease comprising administering a therapeutically effective amount of human erythropoietin protein having the amino acid sequence of SEQ ID NO:1 without administering iron.
2. The method of claim 1, wherein the patient is suffering from heart insufficiency.
3. (Cancelled) The method of claim 1, wherein the erythropoietin protein is epoetin alfa or epoetin beta.
4. (Cancelled) The method of claim 1, wherein the erythropoietin protein has the amino acid sequence of SEQ ID NO:1.
5. ~~(Amended)The method of claim 1, wherein the erythropoietin protein has the sequence of human erythropoietin~~ A method of treating disturbances in iron distribution in a patient suffering from heart disease comprising administering a therapeutically effective amount of human erythropoietin protein having the amino acid sequence of SEQ ID NO:1 modified by the addition of from 1 to 6 up to three glycosylation sites, without administering iron, wherein the modification is selected from the group consisting of:
 - Asn³⁰Thr³².
 - Asn⁵¹Thr⁵³.
 - Asn⁵⁷Thr⁵⁹.
 - Asn⁶⁹.
 - Asn⁶⁹Thr⁷¹.
 - Ser⁶⁸Asn⁶⁹Thr⁷¹.
 - Val⁸⁷Asn⁸⁸Thr⁹⁰.
 - Ser⁸⁷Asn⁸⁸Thr⁹⁰.
 - Ser⁸⁷Asn⁸⁸Gly⁸⁹Thr⁹⁰; (SEQ ID NO: 3);
 - Ser⁸⁷Asn⁸⁸Thr⁹⁰Thr⁹².

Ser⁸⁷Asn⁸⁸Thr⁹⁰Ala¹⁶².
Asn⁶⁹Thr⁷¹Ser⁸⁷Asn⁸⁸Thr⁹⁰.
Asn³⁰Thr³²Val⁸⁷Asn⁸⁸Thr⁹⁰.
Asn⁸⁹Ile⁹⁰Thr⁹¹.
Ser⁸⁷Asn⁸⁹Ile⁹⁰Thr⁹¹.
Asn¹³⁶Thr¹³⁸.
Asn¹³⁸Thr¹⁴⁰.
Thr¹²⁵; and
Pro¹²⁴Thr¹²⁵.

6. (Amended) ~~The method of claim 1, wherein the erythropoietin protein is darbepoetin alfa.~~ A method of treating disturbances in iron distribution in a patient suffering from heart disease comprising administering a therapeutically effective amount of human erythropoietin protein, without administering iron, wherein the protein (EPO) is an analog of SEQ ID NO:1, said analog is selected from the group consisting of: (a) human erythropoietin protein having the amino acid sequence, Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln (SEQ ID NO: 4), extending from the carboxy terminus; (b) the analog in (a) further comprising Ser⁸⁷Asn⁸⁸Thr⁹⁰ EPO; (c) the analog in (a) further comprising Asn³⁰Thr³²Val⁸⁷Asn⁸⁸Thr⁹⁰ EPO; (d) Gln²⁴Ser⁸⁷Asn⁸⁸Thr⁹⁰ EPO; (e) Gln³⁸Ser⁸⁷Asn⁸⁸Thr⁹⁰ EPO; (f) Gln⁸³Ser⁸⁷Asn⁸⁸Thr⁹⁰ EPO and (g) darbepoetin alfa.

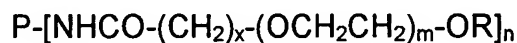
7. (Original) The method of claim 1, wherein the erythropoietin protein is pegylated.

8. (Amended) ~~The A method of treating disturbances in iron distribution in a patient suffering from heart disease comprising administering a conjugate of human erythropoietin protein of SEQ ID NO:1 without administering iron, wherein claim 7,~~ wherein the erythropoietin protein is a conjugate, said conjugate comprising an comprising the erythropoietin protein of SEQ ID NO:1 having one to three free amino groups and having the *in vivo* biological activity of causing bone marrow cells to

~~groups and having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which have a sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; said erythropoietin protein being covalently linked to n poly(ethylene glycol) groups of the formula $-\text{CO}-(\text{CH}_2)_x-(\text{OCH}_2\text{CH}_2)_m-\text{OR}$ with the $-\text{CO}$ of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is lower-alkyl; x is 2 or 3; m is from about 450 to about 900; n is from 1 to 3; and n and m are chosen so that the molecular weight of the conjugate minus the erythropoietin protein is from 20 kilodaltons to 100 kilodaltons.~~

9. (Original) The method of claim 8, wherein x is 2, m is from about 650 to about 750, n is 1 and R is methyl.

10. (Original) The method of claim 8 wherein the conjugate has the formula



wherein

P is the residue of the erythropoietin protein without the free amino group that forms the amide linkage;

R is lower alkyl;

x is 2 or 3;

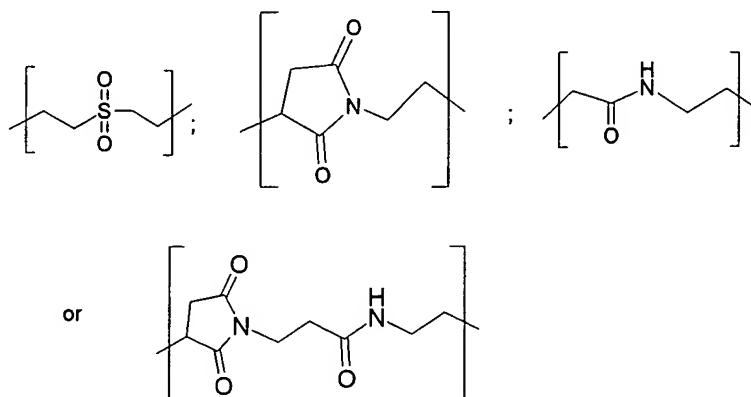
m is from about 450 to about 900; and

n is from 1-3;

and wherein m and n are selected such that the molecular weight of the conjugate minus the erythropoietin protein is from about 20 kd to about 100 kd.

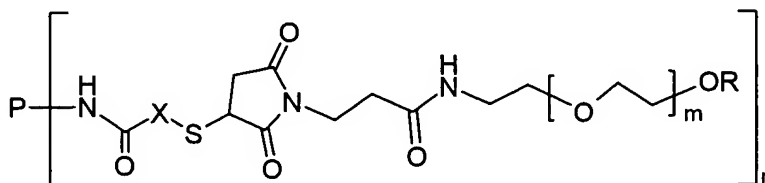
11. (Amended) ~~The method of claim 7, wherein the erythropoietin protein is a conjugate.~~ A method of treating disturbances in a patient suffering from heart disease comprising administering a conjugate of human erythropoietin protein of SEQ ID NO:1.

without administering iron, wherein said conjugate comprising an comprises the
 erythropoietin protein having at least one to three free amino groups and having the in
~~vivo biological activity of causing bone marrow cells to increase production of~~
~~reticulocytes and red blood cells and selected from the group consisting of human~~
~~erythropoietin protein and analogs thereof which have the primary structure of human~~
~~erythropoietin protein modified by the addition of from 1 to 6 glycosylation sites; said~~
~~erythropoietin protein being covalently linked to from one to three lower-alkoxy~~
 poly(ethylene glycol) groups, each poly(ethylene glycol) group being covalently linked to
 the erythropoietin protein *via* a linker of the formula $-C(O)-X-S-Y-$ with the C(O) of the
 linker forming an amide bond with one of said amino groups, X is $-(CH_2)_k-$ or $-$
 $CH_2(O-CH_2-CH_2)_k-$, k is from 1 to 10, Y is



the average molecular weight of each poly(ethylene glycol) moiety is from about 20
 kilodaltons to about 40 kilodaltons, and the molecular weight of the conjugate is from
 about 51 kilodaltons to about 175 kilodaltons.

12. (Original) The method of claim 11, wherein the erythropoietin conjugate has the
 formula:



wherein n is an integer from 1 to 3; m is an integer from 450 to 900; R is lower-alkyl; X is $-(CH_2)_k-$ or $-CH_2(O-CH_2-CH_2)_k-$, k is 1 to 10 and P is the residue of the erythropoietin protein without the n amino groups which form an amide linkage with X.

13. (Amended) The method of claim 1 wherein the amount of human erythropoietin protein administered to the patient is from about 100 U/kg to about 200 U/kg twice per week.

14. (Amended) The method of claim 10 wherein the amount of the human erythropoietin protein administered to the patient is about 200 U/kg once every three weeks.